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Novel Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR Kit (Detection for 3 Genes) Instructions for Use

NON STERILE

REF RR-0479-02 Σ 25 -15℃ -25℃ IVD

For use with ABI Prism®7500/7900; Bio-Rad CFX96; Rotor Gene™6000; SLAN; MIC POC Dx48 Instrument

EC REP Obelis s.a.
Boulevard Général Wahis 53
1030 Brussels, Belgium
Tel: +(32) 2.732.59.54
Fax: +(32) 2.732.60.03
E-Mail : mail@obelis.net

Shanghai ZJ Bio-Tech Co., Ltd.
Building #26, 588 Xinjunhuan Road,
Pujiang High-tech Park, 201114 Shanghai, China
www.liferiverbiotech.com Tel: +86-21-34680598
info@liferiverbiotech.com Fax: +86-21-34680595

1. Intended Use

Novel Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR Kit (Detection for 3 Genes) is used for the in vitro qualitative detection of 2019 novel coronavirus (2019-nCoV) RNA in upper respiratory tract specimens (nasopharyngeal and oropharyngeal extracts) and lower respiratory tract specimens (bronchoalveolar lavage fluid (BALF) and deep cough sputum) by real time PCR systems. It is considered as an aid in the diagnosis of the 2019-nCoV infection.

2. Principle of Real-Time RT-PCR

The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

Real time reverse-transcription polymerase chain reaction (real-time RT-PCR) is used when the starting material is RNA. In this method, RNA is first transcribed into the complementary DNA (cDNA) by reverse transcriptase from total RNA. The cDNA is then used as a template for the real time PCR.

3. Product Description

On January 11, 2020, Chinese health authorities preliminarily identified more than 40 human infections with a novel coronavirus in an outbreak of pneumonia under investigation in Wuhan City, Hubei Province, China. The Chinese authorities identified a new type of coronavirus (novel coronavirus, named as 2019-nCoV), which was isolated on January 7, 2020.

Coronaviruses are a large family of viruses, some causing illness in human and others circulating among animals such as camels, cats and bats. 2019-nCoV is a novel coronavirus. The primer and probe design for this kit is based on the newly released strain (2019-nCoV) (GeneBank accession: MN908947) and covers six 2019-nCoV strains sequences (EPI_ISL_402119, EPI_ISL_402120, EPI_ISL_402121, EPI_ISL_402122, EPI_ISL_402123 and EPI_ISL_402124).

The kit contains a specific ready-to-use system for the detection of Novel Coronavirus (2019-nCoV) RNA by the real-time RT-PCR. The reaction is done in a one-step real time RT-PCR assay in a single tube. It includes a reverse transcription (RT) for the transcription of virus RNA into cDNA and real time PCR for the amplification and detection of the cDNA. Fluorescence is emitted and measured by the real time systems´optical unit during PCR. The detection of amplified virus DNA fragment is performed in fluorimeter **channel FAM, HEX/VIC/JOE** and **Cal Red 610/ ROX/TEXAS RED**.

4. Kit Contents

Ref.	Type of Reagent	Presentation 25rxns
1	Novel CoV (2019-nCoV) Super Mix	1 vial, 513μL
2	RT-PCR Enzyme Mix	1 vial, 27μL
3	Novel CoV (2019-nCoV) Internal Control	1 vial, 30μL
4	Novel CoV (2019-nCoV) Negative Control	1 vial, 400μL
5	Novel CoV (2019-nCoV) Positive Control	1 vial, 30μL

Analytical sensitivity: 1×10^3 copies/mL;

Note: Analytical sensitivity depends on the sample volume, elution volume, nucleic acid extraction method and other factors. If you use the RNA extraction kits recommended, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, the sensitivity can be much higher.

5. Storage

- All reagents should be stored at -20±5℃.
- All reagents can be used till the expiration date indicated on the kit label.
- Repeated thawing and freezing (> 3x) should be avoided as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Super Mix should be stored away from light.

6. Additionally Required Materials and Devices

- Biological cabinet
- Vortex mixer
- Cryo-container
- Sterile filter tips for micro pipets
- Disposable gloves, powderless
- Refrigerator and freezer
- Real time PCR system
- Real time PCR reaction tubes/plates
- Pipets (0.5μL – 1000μL)
- Sterile microtubes
- Biohazard waste container
- Tube racks
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)

7. ⚠Warnings and Precautions

Carefully read this instructions for use before starting the procedure.

- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of reagents as this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the reaction mix on ice or in the cooling block.
- Set up separate working areas for: 1) Reaction setup, 2) Isolation of the RNA and 3) Amplification/detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Discard sample and assay waste according to your local safety regulations.
- Do not pipette by mouth. Do not eat, drink or smoke in laboratory.
- Avoid aerosols

8. Sample Collection, Storage and Transport

- Collect samples in sterile tubes;
- Specimens can be extracted immediately or stored at 2°C~8°C within 24 hours or frozen at -70℃ for long-term.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

9. Procedure

9.1 RNA-Extraction

Different brand RNA extraction kits are available. You may use your own extraction systems or the commercial kits based on the yield. For the RNA extraction, please follow the manufacturer's instructions. The recommended extraction kits are as follows:

Nucleic Acid Isolation Kit	Cat. Number	Manufacturer
RNA Isolation Kit (Paramagnetic Beads Column)	ME-0010	Shanghai ZJ Bio-Tech
RNA Isolation Kit (for Auto-Extraction)	ME-0012	Shanghai ZJ Bio-Tech
Viral RNA Isolation Kit (Preloaded for Auto-Extraction)	ME-0044	Shanghai ZJ Bio-Tech
QIAamp Viral RNA Mini extraction Kit	52904/52906	QIAGEN

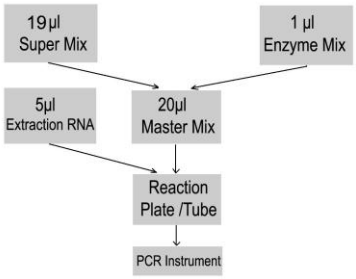
It is noted that the negative control in this kit should be extracted with the same protocol as for specimens. The positive control doesn't need to be extracted with the nucleic acid isolation kit.

9.2 Internal Control

The internal control (IC) in this kit should be added into the extraction mixture with 1μL/test to monitor the whole process.

9.3 RT-PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



1) The volumes of Super Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls and samples prepared. Molecular Grade Water is used as the negative control. For reasons of imprecise pipetting, always add an extra virtual sample. Mix completely and then spin down briefly with a centrifuge.
2) Pipet **20μL** Master Mix with micropipets of sterile filter tips to each of the *Real Time* PCR reaction plate/tubes. Separately add **5μL** template (nucleic acid extracted from negative control and specimen, positive control without extraction) to different reaction plates/tubes. Immediately close the plates/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix and template in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument of ***ABI Prism®7500/7900***; ***Bio-Rad CFX96***; ***Rotor Gene™6000***; ***SLAN***:

45℃ for 10min	1cycle
95℃ for 3min	1cycle
95℃ for 15sec, 58℃ for 30sec (Fluorescence measured at 58℃)	45cycles

Selection of Fluorescence Channels	
FAM	ORF1ab
HEX/VIC/JOE	Gene N
Cal Red 610/ROX/TEXAS RED	Gene E
Cy5	IC

⚠: Perform the following protocol in the instrument *of* ***MIC POC Dx48***:

45℃ for 10min	1cycle
95℃ for 90sec	1cycle
95℃ for 3sec, 58℃ for 20sec (Fluorescence measured at 58℃)	45cycles

Selection of Fluorescence Channels	
Green	ORF1ab
Yellow	Gene N
Orange	Gene E
Red	IC

5) ⚠If you use ABI Prism® system, please choose **"none"** as **passive reference** and **quencher.**

10. Threshold Setting: Just above the maximum level of molecular grade water.

11. Quality Control: Negative Control and Positive Control must be performed correctly; otherwise the sample results are invalid.

Control \ Channel	Ct Value			
	FAM (ORF1ab)	HEX/VIC/JOE (GeneN)	Cal Red 610 (Gene E)	Cy5 (IC)
Negative Control	UNDET	UNDET	UNDET	25~40
Positive Control	≤35	≤35	≤35	≤35

12. Data Analysis and Interpretation

The following sample results are possible:

	Ct Value				Result Analysis
	FAM	HEX/VIC/JOE	Cal Red 610	Cy5	
1#	UNDET	UNDET	UNDET	25~40	Below the detection limit or negative.
2#	⩽40	UNDET	⩽40	——	2019-nCoV positive.
	⩽40	⩽42	UNDET	——	
	⩽40	⩽42	⩽40	——	
	UNDET	⩽42	⩽40	——	
3#	⩽40	UNDET	UNDET	——	Re-test; If Channel FAM is still ⩽ 40 or Channel HEX/VIC/JOE is still ⩽42, report as 2019-nCoV positive.
	UNDET	⩽42	UNDET	——	
4#	UNDET	UNDET	⩽40	——	Re-test; If Channel Cal Red 610 is still ⩽ 40, the specimen might be below the detection limit or other type of coronavirus positive.
5#	UNDET	UNDET	UNDET	UNDET	The result is invalid. Re-test or re-collect the specimen.

For further questions or problems，please contact our technical support at **info@liferiverbiotech.com**